Title: Are northern-edge populations of cork oak more sensitive to drought than those of the southern edge?

Short title: Cork oak responses to drought

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Highlights

- Southern-edge *Q. suber* seedlings invest higher biomass proportion in roots than northern-edge populations.
- Under extreme drought, southern seedlings keep active photosynthesis for longer time than northern ones.
- Root:shoot ratio is directly related to drought resistance.

Abstract

The changes in climate registered at the planetary scale threaten the persistence of current populations for many plant species, with effects particularly evident at the edges of species distributions. However, intraspecific differences in functional traits could modulate the plant responses to the expected increase in drought. Using a trait-based approach, we evaluated under controlled conditions how *Quercus suber* seedlings from the latitudinal edges of the distribution range of the species respond to different watering treatments in terms of vegetative growth and biomass allocation. In addition, we simulated an extreme drought by stopping watering until death to determine chemical and physiological traits under drought stress and to identify which morphological traits were more associated to drought resistance (expressed as survival time without watering). Seedlings from the northern provenance presented higher aboveground biomass allocation (i.e. shoot length and biomass allocation to shoot and leaves), while the southern ones were characterised by longer roots and higher biomass allocation to roots. Under extreme drought, seedlings from the southern provenance maintained higher photosynthetic rates than northern seedlings and were able to modulate their water-use efficiency (estimated from $\delta^{13}\text{C}$) depending on environmental conditions, which allowed them to survive for a longer period. Finally, drought resistance was partially explained by the plant biomass allocation pattern. Traits related to growth in height and light interception were negatively related with drought resistance, whereas traits involving investment in root biomass were positively related with resistance. These geographical differences evidence a local adaptation to drought at the southern edge of *Q. suber* distribution. Our results highlight the importance of the conservation of the genetic resources that peripheral populations harbour at distribution edges.

Keywords: Biomass allocation, functional traits, local adaptation, phenotypic plasticity, photosynthesis, range edge.

Introduction

In the last decade, the registered changes in climate observed at planetary scale are gaining in importance due to its intensity and dramatic consequences on natural populations (IPCC 2013). These climatic variations are inducing strong changes in the probability of establishment and survival of many plant species (Parmesan, 2006; Parmesan and Yohe, 2003; Pérez-Ramos et al., 2013a), with potential repercussions for the maintenance of their current populations (Aubin et al., 2016; Rehm et al., 2015). However, these changes do not likely affect homogeneously species across their geographical ranges, with populations located at the edges of their distributions being especially sensitive to climatic alterations as conditions are already at the species' tolerance limit (Andreu et al. 2007; Linares and Tíscar 2011; Matías et al. 2017). In consequence, it has been suggested by numerous observational (Jump et al., 2006; Matías and Jump, 2015), experimental (Bombi et al., 2017; Merlin et al., 2018) and modelling studies (Thuiller, 2003; Vessella et al., 2017) that current distribution limits of major tree species will be considerably modified along the next decades.

Beside the existing solid evidence for climatic alterations of species distribution ranges, the response of edge populations to climate change can be modulated by different mechanisms. Plants have the capacity to cope with environmental changes by means of variations in different suites of traits (Bussotti, 2008; Freschet et al., 2018; Reich et al., 2007). Higher intraspecific variability in some key traits increases the probability of plant species to survive under different climate change scenarios, since the likelihood to obtain phenotypes tuned for the new conditions is improved (Benito-

Garzón et al., 2011). This variability can be obtained from two main sources. On the one hand, phenotypic plasticity represents the range of different phenotypes that a single genotype can express depending on the environmental conditions (Bongers et al., 2017; Garland and Kelly, 2006; Nicotra et al., 2010). It allows plastic responses to fast changes in climate, and is essential for plant acclimation (Bussotti et al., 2015). But the acclimation capacity reaches a limit when the environmental pressure exceeds the intrinsic capacity of the plant for the variation of fundamental adaptive traits (Chevin et al., 2010; Thuiller et al., 2008), which can occur at the edges of species' distributions. On the other hand, local adaptation is the result of the natural selection of genotypes better fitted to the local environment. The fixation of locally-adapted traits entails a change in the genome through evolutionary processes, and requires some type of population isolation by means of geographical barriers to pollen and seed dispersal (Savolainen, Pyhäjärvi, and Knürr 2007; Ghannoum and Way 2011). The long exposure of rear-edge populations to xeric conditions can result in local adaptations to drought stress (Matías et al., 2014), and such populations might represent important genetic reservoirs holding drought-resistant genotypes. Thus, although aridity is expected to be more intense at the rear edge, it is not clear whether local adaptations might mitigate its impacts to some extent. The relative importance of phenotypic plasticity and local adaptation as functional mechanisms to cope with drought remains largely underexplored in the context of climate change (Aubin et al., 2016; Dawson et al., 2011). The identification of plant populations and genotypes with high tolerance to drought might become particularly relevant to improve our predictive capacity and implement management strategies to deal with the increasing aridity expected for the coming decades.

The functional approach (Lavorel and Garnier, 2002) has been developed during

the last years as a powerful tool to quantify variations in the morphological and physiological plant traits that could be driving the response of plant species and communities to variations in environmental conditions (Bussotti et al., 2015; Lloret et al., 2013; Pérez-Ramos et al., 2017). The analysis of the variability in key functional traits across contrasted resource-availability and geographical gradients can be useful to explore plastic and adaptive responses to cope with the expected environmental changes, as well as to infer their potential consequences for species distributions (Sniderhan, McNickle, and Baltzer 2018; Savolainen et al. 2004). In consequence, gaining knowledge about the variation of functional trait values among and within geographically-distant populations of a given species, together with the identification of those traits more associated to stress tolerance, opens the possibility for a mechanistic assessment of species vulnerability to climate change (Aubin et al., 2016; Bussotti et al., 2015).

The long-life span and low migration rate of tree species makes them particularly sensitive to strong changes in climate (Jump and Peñuelas, 2005), especially in very vulnerable areas as the Mediterranean region (Giorgi and Lionello, 2008). Among Mediterranean trees, cork oak (*Quercus suber* L., Fagaceae) is a keystone species dominating stands characterised by their high biodiversity and ecological value, and represents a valuable economic resource for many countries in the Mediterranean basin (Aronson et al., 2009; Ibáñez et al., 2014). However, its natural populations are currently under dramatic declining trends characterised by high adult mortality rates and low regeneration capacity (Camilo-Alves et al., 2013; Ibáñez et al., 2017; Pérez-Ramos et al., 2012). These processes are especially evident at the southern part of the species' latitudinal distribution (Matías et al., 2019), and have been attributed to a combination of drought stress and infection by the aggressive exotic pathogen *Phytophthora*

cinnamomi (Camilo-Alves et al., 2017; Costa et al., 2010; Gómez-Aparicio et al., 2012; Matías et al., 2019). Based on the previous evidence, we hypothesised that *Q. suber* populations from the equatorial edge of the distribution have developed drought adaptations due to their longer persistence in xeric areas, harbouring drought-resistant genotypes which make them less vulnerable to the expected drought increase than populations from the northern limit of the distribution. To test this hypothesis, we performed an experiment under controlled conditions where *Q. suber* seedlings from both latitudinal extremes of the species' distribution were grown under three different watering levels and subsequently subjected to an extreme drought until death. We used a trait-based approach to characterize seedling responses to water variability in terms of vegetative growth and biomass allocation, to explore the chemical and physiological responses to drought stress, and to identify which morphological traits were more associated to drought resistance (expressed as survival time without watering). The quantification of trait variations across different watering and provenance levels allowed us to determine phenotypic and genotypic responses to environmental alterations, respectively. Specifically, we aimed to answer the following questions: *i*) Which are the most plastic traits in response to a reduction in water availability during seedling development? *ii*) Are there differences in the response to drought between *Q. suber* populations from the two latitudinal edges of the distribution? In that case, *iii*) which traits are driving the differential drought resistance between the two extremes of the distribution?

Materials and methods

Focal species and seed sources

Cork oak (*Quercus suber* L.) was selected as focal species for this study given its ecological and economic importance in Mediterranean forests. *Q. suber* is a sclerophyllous evergreen oak species inhabiting the western Mediterranean region, being especially abundant in the Atlantic coast of the Iberian Peninsula. To determine local adaptations to drought, we selected two populations, one at each latitudinal extreme of the species distribution. From November to December 2015, mature acorns were collected from populations at the northern (Sobreiral do Arnego, NW Spain) and the southern (Tazekka National Park, N Morocco) range limits of the species (Table S1), selecting five trees per population separated by at least 50 m and 50 acorns per tree. Acorns were tested for viability by the flotation method (Gribko and Jones, 1995) and stored in wet vermiculite at 5 °C until sowing.

Experimental design

The experiment was conducted at the greenhouse facilities of La Hampa experimental station (IRNAS-CSIC), located at Coria del Río (Seville, Spain; 37° 17' 01" N; 6° 03' 57" W) from 22 January to 9 December 2016 (46 weeks). This period covered the first year of life of tree seedlings, the most critical stage in the ontogeny of Mediterranean woody species (Matías et al., 2011; Pérez-Ramos and Marañón, 2012). In January 2016, 300 tubular PVC pots (12 cm in diameter and 50 cm height) were filled with a peat and river sand mixture (proportion 2:1 by volume). To allow mycorrhization, all pots were irrigated with 300 ml of a soil microbial inoculum obtained from the maceration 10 kg of soil collected under the canopy of *Q. suber* on 100 l of water for 48 h (Matías and Jump, 2014). This soil was collected from a population central to both seed sources (Doñana National Park, SW Spain) and that was analysed to confirm the absence of the soil-borne pathogen *P. cinnamomi* following the protocol of Sánchez et al. (2002). One

individual acorn was sown per pot at 2 cm depth (30 acorns per mother tree, 150 per provenance in total), and all experimental pots were kept under optimal irrigation until 85 % of the seedlings emerged, which occurred after 7 weeks (March 2016). In order to determine the role of different precipitation scenarios during seedling growth, after this initial phase of non-limiting water supply we simulated three different precipitation scenarios: 1) *High water availability*: corresponding with a weekly irrigation until field capacity, and equivalent to a rainy spring where soils remain saturated most of the time; 2) *Medium water availability*: corresponding with a reduction of 40 % with respect to the previous level, simulating average spring conditions during the growing season (Pérez-Ramos and Marañón, 2012); 3) *Low water availability*: watering amount reduced 70 % with respect to the high water availability treatment, simulating a dry spring. These watering treatments were applied by adding a constant amount of water to all pots within the same watering level between the experimental weeks 8 to 20 (15 June 2016), simulating the natural growing season.

At this point, we split the experiment in two groups by randomly selecting half of the individuals per treatment combination. One of the groups was harvested for the determination of population and morphological responses to watering reduction during early growth (see below). To determine the resistance to extreme drought of seedlings growing under the different watering and provenance treatments, the other group was subjected to a progressive drought until death with no watering from this point onwards, simulating an extreme drought period. Light levels in the greenhouse mimicked the light conditions of forest understorey (PAR ca. 300 $\mu\text{mol m}^{-2} \text{s}^{-1}$) with natural daily duration. Temperature remained constant between 25 °C and 30 °C throughout the experiment. Survival was weekly monitored until all seedlings died. Seedlings were progressively harvested as they died. Soil moisture was measured fortnightly during the application of

the watering treatments, and weekly during the extreme drought period using a TDR probe (Easy-Test Technologies, Poland) on the top 10 cm. The position of the 300 pots was rotated bi-weekly to avoid the effect of possible small differences in environmental conditions.

Determination of physiological, chemical and morphological traits in response to extreme drought

A random subset of five individuals per provenance and watering combination, including one per mother tree, was selected for the determination of several physiological traits. From this subset, we weekly collected one leaf per seedling between the experimental weeks 22 and 28 (22 June to 3 August) for the determination of the leaf relative water content (LRWC) during the period of extreme drought. These measurements were used as a proxy of plant water status (Pérez-Ramos et al., 2013b). Collected leaves were quickly sealed into pre-weighed vials. The difference in the weight of the filled and empty vials provided the fresh weight (FW). The vials were filled with distilled water and refrigerated in darkness at 4 °C for 24 hours to allow for leaf rehydration. Hydrated leaves were then removed from the vials, patted surface dry, and weighed, providing full turgor weight (TW). Next, the leaves were placed into open vials and oven-dried at 60 °C for 48 hours. Upon removal, the vials were immediately sealed and weighed. The contents were discarded and the empty vials weighed. The difference between weights provided the dry weight (DW). LRWC (%) was calculated using the formula: $LRWC = 100 \times [(FW-DW)/(TW-DW)]$. Maximum leaf-level photosynthesis (A_{max} , $\mu\text{mol m}^{-2} \text{s}^{-1}$) was measured on the same individuals used for LRWC measurements once before the start of the period of extreme drought (March, week 16) and weekly along this period (i.e. June-July, experimental weeks 21 to 27)

following the method described in Appendix A. These five seedlings per treatment combination were also used for isotopic discrimination analyses at the end of the extreme drought period. After death, leaves were ground to powder and total carbon and nitrogen as well as the bulk isotopic composition ($\delta^{13}\text{C}$) were analysed as described in Appendix A. The C:N ratio was calculated from these primary data. These variables inform about the plant water stress (LRWC), the maximum capacity to fix carbon (A_{max}), the plant nutrition status (C:N) and the efficiency in the use of water ($\delta^{13}\text{C}$). The

For both harvesting times (after the application of the watering treatments and after death following the extreme drought period), seedlings were separated into stem, leaf and root fractions, measuring maximum length of the shoot and root in all plants. From a random subset of five individuals per provenance and watering combination (one per mother tree), we collected seven leaves per individual and scanned them for the determination of leaf area using the software Image-Pro Plus v 4.5 (Media Cybernetics, Silver Spring, MD, USA). Then, all samples were oven-dried at 60 °C for 72 h in order to obtain constant weight. From these primary data, a set of morphological traits was calculated following Pérez-Harguindeguy et al. (2013) recommendations: total biomass (in g), root:shoot ratio (R:S; root mass/aboveground mass, g g⁻¹), leaf mass ratio (LMR; leaf mass/total plant mass, g g⁻¹), stem mass ratio (SMR; stem mass/total plant mass, g g⁻¹), specific leaf area (SLA; total leaf area/total leaf mass, m² kg⁻¹) and specific stem length (SSL; stem length/stem mass, cm g⁻¹). These variables refer, respectively, to biomass allocation (R:S, SMR, LMR), leaf display (SLA) and the efficiency of biomass investment for height gain (SSL) (Poorter, 1999).

Data analysis

Variations among treatments in soil moisture during the experiment, and in *Amax* and LRWC during the extreme drought period were tested using repeated-measures analysis of variance (rm-ANOVA) with provenance and watering level as between-subject factors and time as a within-subject factor. Linear Models including watering treatment, provenance and its interactions as fixed factors were used to analyse differences in morphological traits using the data of the first harvest (before the start of the extreme drought period). The same modelling approach was used to test for differences in $\delta^{13}\text{C}$ and leaf C:N but using data from the second harvest (i.e. seedlings subjected to extreme drought). The effect of the mother tree on morphological traits was also tested, but finally excluded from analyses given its lack of significance in all cases. Given the reduced sample size, it was not possible to analyse the maternal effect for physiological and chemical traits. Differences among watering levels and provenances in seedling survival during the extreme drought period (i.e. without watering) were analysed using a failure-time approach, which measures the time to death of each individual seedling (defined as the number of days without watering until death), using a Log-Rank test to estimate differences among curves (Fox, 2001). Survival time during the extreme drought period was also used as an indicator of drought resistance, and was related by simple regression to the different morphological traits of the same individual to look for significant associations. Transformations to assure normality and homoscedasticity of data were used when necessary. All analyses were performed in R (R Development Core Team, 2016). All values are given as mean $\pm$ SE.

Results

Soil moisture

Watering levels applied during the experiment resulted in different values of soil moisture across treatments, decreasing from high to medium and low watering levels during the whole treatment application (38.6 ± 2.2 %, 31.0 ± 2.1 % and 27.5 ± 1.9 %, respectively; $F_{2,121} = 471.2$, $P < 0.0001$). These differences remained after watering stopped in week 20 for 12 more weeks (Fig. S1). By contrast, there were no differences in soil moisture between pots containing seedlings from the northern or southern provenances ($F_{2,121} = 0.9$, $P = 0.3$).

Morphological responses to different watering levels

Survival was 100 % in all treatment combinations during the watering stage, and there were no differences among treatments in total biomass (Table 1). However, we found strong effects of both provenance and watering treatments on the different morphological traits measured. Seedlings from the northern provenance presented higher shoot length, SLA and LMR, whereas seedlings from the southern provenance showed higher root length, R:S ratio and SSL (Table 1, S2). Across watering levels, high water availability increased root investment (higher R:S ratio), whereas low water availability increased SLA values (Fig. 1). Shoot length, SMR and LMR were highest under the medium watering level (Table S2). There were no significant interactions between provenance and watering for any of the morphological traits measured.

Physiological and chemical responses to extreme drought

Although there was a clear trend across watering levels, differences became significant only at the end of the experiment (week 28) as leaves dried. As expected, *Q. suber* seedlings diminished their leaf water content (LRWC) in response to progressive drought. Although they were able to maintain high leaf saturation during the application

of the watering levels, leaves started to lose turgor after four weeks without water supply (Fig. 2). Seedlings grown under the high watering level kept higher LRWC values, whereas seedlings grown under the low watering treatment experienced a faster decrease of LRWC. Northern and southern provenances lost LRWC with increased drought at a similar rate (Fig. 2; Table S3).

In parallel to leaf dehydration, seedlings immediately started to diminish their photosynthetic activity after the extreme drought imposition (Fig. 3). Although there were no differences among treatments during the watering stage (week 16), differences in A_{max} appeared as soil (and leaves) dried (Table S3). The high watering treatment allowed a significantly higher photosynthetic activity for a longer period, while A_{max} was equally reduced under the medium and low watering levels. But more interestingly, although both provenances maintained a similar photosynthetic rate with no significant differences when the whole sampling period was considered (Table S3), significant differences in A_{max} between provenances appeared within specific sampling dates (Fig. 3) when soil moisture dropped below 5 % (Fig. S1). Under extreme drought (weeks 26-27), seedlings from the southern provenance maintained higher A_{max} and continued gaining carbon, while seedlings from the northern provenance presented negative A_{max} values, indicating that respiration predominated over photosynthesis.

We found no differences between provenances in the leaf C:N ratio ($F = 0.32$, $P = 0.58$, $df = 1$), but the watering treatment significantly altered this ratio ($F = 23.4$, $P < 0.0001$, $df = 2$; Fig. 4a), with a N enrichment in leaves under low or medium watering levels (Table S2). Seedlings from the northern provenance did not present differences across watering levels in $\delta^{13}C$ (Fig. 4b). However, seedlings from the southern edge of the distribution showed less negative $\delta^{13}C$ values as water availability was reduced (provenance: $F = 1.29$, $P = 0.27$, $df = 1$; watering: $F = 4.75$, $P = 0.02$, $df = 2$). We

detected a marginal interaction between provenance and watering in $\delta^{13}\text{C}$ ($F = 2.64$, $P = 0.09$, $df = 2$).

Drought resistance and its relation with morphological traits

There were differences in survival time after extreme drought between provenances ($\chi^2 = 6.9$, $P = 0.009$, $df = 1$) and watering levels ($\chi^2 = 27.0$, $P < 0.0001$, $df = 2$). Seedlings from the southern provenance showed longer survival times (74 ± 6 days) than those coming from the northern provenance (53 ± 4 days). Regarding watering treatments, seedlings subjected to high water availability during the watering period showed higher survival during the extreme drought period (86 ± 6 days) than seedlings from medium (57 ± 5 days) and low watering levels (46 ± 5 days, Fig. S2). We detected a significant correlation between the number of surviving days since the onset of extreme drought (an indicator of drought resistance) and some morphological traits. As a general rule, the trait related to root investment (R:S ratio: $R^2 = 0.30$, $P < 0.0001$) was positively related to drought resistance (Fig. 5), whereas individuals with higher values in traits maximising aboveground development (SMR: $R^2 = 0.38$, $P < 0.0001$; LMR: $R^2 = 0.17$, $P < 0.0001$) died faster, indicating lower drought resistance.

Discussion

In this study we experimentally determined the responses to different water availability levels and to a progressive drought during the early development of *Q. suber* seedlings using seeds from the latitudinal edges of the species' distribution. We detected morphological and physiological differences in the response to drought between the two geographical limits. Seedlings from the southernmost distribution developed better their root system, allowing them to gain carbon for a longer period and to present higher

water use efficiency, which ultimately translated into a higher resistance to extreme drought.

Trait plasticity in response to watering

Although differential water availability during the growing season had no effect on the photosynthetic capacity and total biomass of *Q. suber* seedlings, the biomass allocation pattern showed a very plastic response, resulting in differences in morphological traits across watering levels. As a general rule, those traits maximising aboveground growth (i.e. shoot length, LMR) were higher at medium watering levels. It has been proven in previous studies with oak species that either an excess of water or drought conditions reduce seedling development (Pérez-Ramos and Marañón 2009; Gómez-Aparicio et al. 2008; Quero et al. 2008), while intermediate moisture levels without the hypoxic or water scarcity limitations could allow a better development of aerial traits. Under optimal watering conditions, higher development of the aboveground part provides several benefits for seedlings, such as self-shading avoidance or competitive advantages (Mediavilla and Escudero, 2010; Saha et al., 2014). SLA is a key trait expressing the trade-off between light interception and water loss, and is commonly assumed to increase under shaded and moist conditions (Aranda et al., 2005; Poorter and Markesteijn, 2007). However, we found the highest SLA values under the driest scenario simulated in our experiment. Although this result could seem rather counterintuitive, it could be an indirect consequence of the higher N concentration reported in the low water availability treatment (Table S2), since SLA is usually correlated with leaf N (Wright et al., 2004). Also contrary to our expectations, *Q. suber* seedlings devoted a higher proportion of biomass to roots (i.e. higher R:S ratio) under the high water availability treatment. It is usually assumed that higher root development

is a common response to water and nutrient limitation (Matías et al., 2016; Padilla et al., 2009), which allows for a better exploration of soil and resource uptake. Further studies are therefore needed to find the mechanisms explaining the trends reported in our experiment.

Geographic differences in the response to drought

Our results detected differences in morphological and physiological traits between the two latitudinal edges of the distribution. After the watering period, seedlings from the northern limit clearly invested a higher proportion of their biomass on aboveground development, presenting higher values for traits such as shoot length, SMR, LMR or SLA. This higher aerial growth has clear benefits for light capture and competition with other understory species (Saha et al., 2014), and is a typical strategy from humid environments where water or nutrients are not the limiting factors. Thus, at the northern edge of *Q. suber*, where precipitation is high throughout the year and soils are rich in nutrients (Sánchez, García-Sánchez, and Dios 1967), the fixation of those traits related to improve aboveground competitive abilities might be a good strategy to increase recruitment. On the contrary, seedlings from the southern provenance consistently presented higher values in those traits related to root development (root length and R:S ratio). A more developed radicular system allows for a better exploration of deeper soil layers for water and nutrient uptake (Nicotra, 2002; Paula and Ojeda, 2009), and has been identified as a key trait related to drought resistance (Markestijn and Poorter, 2009; Matías et al., 2014; Pérez-Ramos et al., 2013b). In consequence, our results suggest a clear selective pressure for opposite values in morphological traits across *Q. suber* latitudinal distribution aimed to maximise the uptake of the most limiting resource, which resulted in local adaptations for aboveground competence or soil

exploration at northern and southern range edges, respectively. However, given the low number of mother trees used in this study, it would be necessary to explore the trait variability within natural populations to determine the effective prevalence of local adaptations.

In addition to these differences in morphological traits, *Q. suber* seedlings from the latitudinal edges of the distribution also presented different physiological values under extreme drought. Although initially similar, the photosynthetic capacity (A_{max}) of seedlings from both provenances started to differentiate under a prolonged water stress (Fig. 3). The lack of differences in A_{max} or in LRWC during the watering period indicates that the low water availability treatment was not severe enough to induce water stress during the growing period. Soil desiccation reduced A_{max} similarly for both provenances but, when soil water availability was very low, seedlings from the northern edge strongly diminished photosynthesis, reaching even negative values. Negative A_{max} values imply that under extreme drought leaf respiration predominates over photosynthesis (Gallé et al., 2007), and there is a net loss of carbon leading to carbon starvation and seedling death (McDowell et al., 2008). In contrast, seedlings from the southern edge of the distribution were able to maintain positive A_{max} values with the same water availability, continuing the carbon gain over a large part of the drought period, which allowed them to survive for a longer period. This result directly relates to the pattern found in the carbon isotopic discrimination analyses. Less negative $\delta^{13}C$ values are directly related to a higher capacity to fix carbon per unit of water lost (i.e. the intrinsic water use efficiency, iWUE; Farquhar, Ehleringer, and Hubick 1989). Although the interaction between provenance and watering was only marginally significant (probably due to the low sample size for these analyses, $N = 5$), seedlings from northern and southern provenances presented different isotopic discrimination. We

found no differences across watering levels in seedlings from the northern provenance, while southern seedlings increased $\delta^{13}\text{C}$ values as watering diminished (Fig. 4). This result implies that seedlings from the southern provenance have the capacity to modulate iWUE depending on environmental conditions and to buffer the effects of the predicted climate to some extent by maintaining active photosynthesis (higher A_{max} , see above) for a longer period. On the contrary, iWUE seems to be fixed in *Q. suber* seedlings from the northern edge of the distribution, showing a very low plasticity in this trait. In consequence, the lower capacity of acclimation of seedlings from the northern distribution might make them more sensitive to the expected increase in drought frequency and intensity predicted by climate change models.

The role of traits as drivers of drought resistance

The results from this study show that several morphological traits could be useful to predict plant responses to drought. Thus, drought resistance, expressed as the survival time without watering, was partially explained by the plant biomass allocation pattern. Traits related to growth in height (SMR) and light interception (LMR) were negatively related to drought resistance, while plants maximising soil exploration (higher R:S values) survived longer. A higher development of the root system allows seedlings to forage more efficiently a higher soil volume and deeper soil layers, where more water is usually available (Engelbrecht et al., 2005), leading to higher survival. Greater aboveground development represents a clear advantage when light is the limiting factor (Green and Juniper, 2004; Mediavilla and Escudero, 2010) but, at the same time, it increments the evaporative demands of the plant. Consequently, a reduction in LMR helps to diminish the amount of transpirational tissue and thus to minimise water loss (Poorter and Markesteijn, 2007). Our results confirm that a higher proportion of root

biomass increases seedling survival under drought stress by simultaneously increasing water uptake and diminishing the evaporative surface. These results support previous studies indicating that individuals originated in drought-prone locations or from equatorial distributions present higher survival rates in dry years (Ramírez-Valiente et al. 2009; Matías, González-Díaz, and Jump 2014; Kolb et al. 2016), and provide a mechanistic explanation about the traits implied in drought resistance.

Conclusions

Our results show evidence for phenotypic plasticity in the response of *Q. suber* seedlings to different levels of water availability. This plasticity allows the uptake of resources when they are available, as it is the case of rainy spring seasons, enhancing survival probabilities during summer drought. More interestingly, we detected geographical differences between the latitudinal edges of the species' distribution in terms of the biomass allocation patterns, with higher aerial growth in northern seedlings and larger belowground development at the southern edge. This differential growth pattern, in addition to the higher photosynthetic capacity under extreme drought and the ability to modulate iWUE depending on water availability, resulted in a higher drought resistance at the southern distribution limit. Although there were differences in morphology between provenances in early growth, the greater physiological differences (i.e. distinct ability to gain carbon) appeared under extreme drought conditions. These geographical differences, together with the lack of maternal effects within provenances, seem to indicate that these traits have been fixed at population level, and suggest a local adaptation to drought at the southern edge of *Q. suber* distribution.

Under a global change scenario, where extreme drought events are expected to increase in frequency, the role that intraspecific genetic diversity might play in the

response capacity of tree populations to environmental alterations is gaining in importance. Our results highlight the importance of the conservation of genetic resources that peripheral populations harbour at distribution edges. From an applied perspective, the higher drought resistance of southern-edge populations would represent a valuable resource for the restoration of degraded forests or in areas suffering tree decline in order to increase their resilience to climate change. However, the consequences that assisted species migration can have on the mid- to long-term are not still clear enough (see McLachlan, Hellmann, and Schwartz, 2007 or Benito-Garzón et al., 2018 for a debate).

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References

- Andreu, L., Gutiérrez, E., Macias, M., Ribas, M., Bosch, O., Camarero, J.J., 2007. Climate increases regional tree-growth variability in Iberian pine forests. *Glob. Chang. Biol.* 13, 804–815. <https://doi.org/10.1111/j.1365-2486.2007.01322.x>
- Aranda, I., Castro, L., Pardos, M., Gil, L., Pardos, J.A., 2005. Effects of the interaction between drought and shade on water relations, gas exchange and morphological traits in cork oak (*Quercus suber* L.) seedlings. *For. Ecol. Manage.* 210, 117–129. <https://doi.org/10.1016/J.FORECO.2005.02.012>
- Aronson, J., Pereira, J.S., Pausas, J.G., Society for Ecological Restoration International., 2009. Cork oak woodlands on the edge : ecology, adaptive management, and restoration. Island Press.
- Aubin, I., Munson, A.D., Cardou, F., Burton, P.J., Isabel, N., Pedlar, J.H., Paquette, A., Taylor, A.R., Delagrange, S., Kebli, H., Messier, C., Shipley, B., Valladares, F., Kattge, J., Boisvert-Marsh, L., McKenney, D., 2016. Traits to stay, traits to move: a review of functional traits to assess sensitivity and adaptive capacity of temperate

and boreal trees to climate change. *Environ. Rev.* 24, 164–186.
<https://doi.org/10.1139/er-2015-0072>

Benito-Garzón, M., Fady, B., Davi, H., Vizcaíno-Palomar, N., Fernández-Manjarrés, J.,
2018. Trees on the move: using decision theory to compensate for climate change
at the regional scale in forest social-ecological systems. *Reg. Environ. Chang.* 18,
1427–1437. <https://doi.org/10.1007/s10113-018-1277-y>

Benito Garzón, M., Alía, R., Robson, T.M., Zavala, M.A., 2011. Intra-specific
variability and plasticity influence potential tree species distributions under climate
change. *Glob. Ecol. Biogeogr.* 20, 766–778. <https://doi.org/10.1111/j.1466-8238.2010.00646.x>

Bombi, P., D’Andrea, E., Rezaie, N., Cammarano, M., Matteucci, G., 2017. Which
climate change path are we following? Bad news from Scots pine. *PLoS One* 12,
e0189468. <https://doi.org/10.1371/journal.pone.0189468>

Bongers, F.J., Olmo, M., Lopez-Iglesias, B., Anten, N.P.R., Villar, R., 2017. Drought
responses, phenotypic plasticity and survival of Mediterranean species in two
different microclimatic sites. *Plant Biol.* 19, 386–395.
<https://doi.org/10.1111/plb.12544>

Bussotti, F., 2008. Functional leaf traits, plant communities and acclimation processes
in relation to oxidative stress in trees: a critical overview. *Glob. Chang. Biol.*
<https://doi.org/10.1111/j.1365-2486.2008.01677.x>

Bussotti, F., Pollastrini, M., Holland, V., Brüggemann, W., 2015. Functional traits and
adaptive capacity of European forests to climate change. *Environ. Exp. Bot.* 111,
91–113. <https://doi.org/10.1016/j.envexpbot.2014.11.006>

Camilo-Alves, C., da Clara, M.I.E., de Almeida, N.M.C., 2013. Decline of
Mediterranean oak trees and its association with *Phytophthora cinnamomi*: a
review. *Eur. J. For. Res.* 132, 411–432. <https://doi.org/10.1007/s10342-013-0688-z>

Camilo-Alves, C., Vaz, M., Da Clara, M.I.E., Ribeiro, N.M., 2017. Chronic cork oak
decline and water status: new insights. *New For.* 48, 753–772.
<https://doi.org/10.1007/s11056-017-9595-3>

Chevin, L.-M., Lande, R., Mace, G.M., 2010. Adaptation, Plasticity, and Extinction in a
Changing Environment: Towards a Predictive Theory. *PLoS Biol.* 8, e1000357.
<https://doi.org/10.1371/journal.pbio.1000357>

Costa, A., Pereira, H., Madeira, M., 2010. Analysis of spatial patterns of oak decline in
cork oak woodlands in Mediterranean conditions. *Ann. For. Sci.* 67, 204–204.
<https://doi.org/10.1051/forest/2009097>

Dawson, T.P., Jackson, S.T., House, J.I., Prentice, I.C., Mace, G.M., 2011. Beyond
Predictions: Biodiversity Conservation in a Changing Climate. *Science* (80-.).
332, 53–58.

Engelbrecht, B.M.J., Kursar, T.A., Tyree, M.T., 2005. Drought effects on seedling
survival in a tropical moist forest. *Trees* 19, 312–321.
<https://doi.org/10.1007/s00468-004-0393-0>

- 543 Farquhar, G.D., Ehleringer, J.R., Hubick, K.T., 1989. Carbon isotope discrimination and
544 photosynthesis. *Annu. Rev. Plant Physiol. Plant Mol. Biol.* 40, 503–540.
- 545 Fox, G., 2001. Failure time analysis: studying times-to-events and rates at which events
546 occur, in: S.M. Scheiner, Gurevitch, J. (Eds.), *Design and Analysis of Ecological*
547 *Experiments*. Oxford University Press, New York, pp. 235–266.
- 548 Freschet, G.T., Violle, C., Bourget, M.Y., Scherer-Lorenzen, M., Fort, F., 2018.
549 Allocation, morphology, physiology, architecture: the multiple facets of plant
550 above- and below-ground responses to resource stress. *New Phytol.* 219, 1338–
551 1352. <https://doi.org/10.1111/nph.15225>
- 552 Gallé, A., Haldimann, P., Feller, U., 2007. Photosynthetic performance and water
553 relations in young pubescent oak (*Quercus pubescens*) trees during drought stress
554 and recovery. *New Phytol.* 174, 799–810. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2007.02047.x)
555 [8137.2007.02047.x](https://doi.org/10.1111/j.1469-8137.2007.02047.x)
- 556 Garland, T., Kelly, S.A., 2006. Phenotypic plasticity and experimental evolution. *J.*
557 *Exp. Biol.* 209, 2344–2361. <https://doi.org/10.1242/jeb.022673>
- 558 Ghannoum, O., Way, D.A., 2011. On the role of ecological adaptation and geographic
559 distribution in the response of trees to climate change. *Tree Physiol.* 31, 1273–
560 1276. <https://doi.org/10.1093/treephys/tpr115>
- 561 Giorgi, F., Lionello, P., 2008. Climate change projections for the Mediterranean region.
562 *Glob. Planet. Change* 63, 90–104. <https://doi.org/10.1016/j.gloplacha.2007.09.005>
- 563 Gómez-Aparicio, L., Ibáñez, B., Serrano, M.S., De Vita, P., Ávila, J.M., Pérez-Ramos,
564 I.M., García, L. V., Esperanza Sánchez, M., Marañón, T., 2012. Spatial patterns of
565 soil pathogens in declining Mediterranean forests: Implications for tree species
566 regeneration. *New Phytol.* 194, 1014–1024. [https://doi.org/10.1111/j.1469-](https://doi.org/10.1111/j.1469-8137.2012.04108.x)
567 [8137.2012.04108.x](https://doi.org/10.1111/j.1469-8137.2012.04108.x)
- 568 Gómez-Aparicio, L., Pérez-Ramos, I.M., Mendoza, I., Matías, L., Quero, J.L., Castro,
569 J., Zamora, R., Marañón, T., 2008. Oak seedling survival and growth along
570 resource gradients in Mediterranean forests: Implications for regeneration in
571 current and future environmental scenarios. *Oikos* 117, 1683–1699.
572 <https://doi.org/10.1111/j.1600-0706.2008.16814.x>
- 573 Green, P.T., Juniper, P.A., 2004. Seed-seedling allometry in tropical rain forest trees:
574 seed mass-related patterns of resource allocation and the ‘reserve effect.’ *J. Ecol.*
575 92, 397–408. <https://doi.org/10.1111/j.0022-0477.2004.00889.x>
- 576 Gribko, L.S., Jones, W.E., 1995. Test of the float method of assessing northern red oak
577 acorn condition. *Tree Plant. Notes* 46, 143–147.
- 578 Ibáñez, B., Gómez-Aparicio, L., Ávila, J.M., Pérez-Ramos, I.M., Marañón, T., 2017.
579 Effects of *Quercus suber* Decline on Woody Plant Regeneration: Potential
580 Implications for Successional Dynamics in Mediterranean Forests. *Ecosystems* 20,
581 630–644. <https://doi.org/10.1007/s10021-016-0044-5>
- 582 Ibáñez, B., Ibáñez, I., Gómez-Aparicio, L., Ruiz-Benito, P., García, L. V., Marañón, T.,
583 2014. Contrasting effects of climate change along life stages of a dominant tree
584 species: The importance of soil-climate interactions. *Divers. Distrib.* 20, 872–883.

585 <https://doi.org/10.1111/ddi.12193>

586 Jump, A.S., Hunt, J.M., Peñuelas, J., 2006. Rapid climate change-related growth decline
587 at the southern range edge of *Fagus sylvatica*. *Glob. Chang. Biol.* 12, 2163–2174.
588 <https://doi.org/10.1111/j.1365-2486.2006.01250.x>

589 Jump, A.S., Penuelas, J., 2005. Running to stand still: adaptation and the response of
590 plants to rapid climate change. *Ecol. Lett.* 8, 1010–1020.
591 <https://doi.org/10.1111/j.1461-0248.2005.00796.x>

592 Kolb, T.E., Grady, K.C., McEttrick, M.P., Herrero, A., 2016. Local-Scale Drought
593 Adaptation of Ponderosa Pine Seedlings at Habitat Ecotones. *For. Sci.* 62, 641–
594 651. <https://doi.org/10.5849/forsci.16-049>

595 Lavorel, S., Garnier, E., 2002. Predicting changes in community composition and
596 ecosystem functioning from plant traits: revisiting the Holy Grail. *Funct. Ecol.* 16,
597 545–556. <https://doi.org/10.1046/j.1365-2435.2002.00664.x>

598 Linares, J.C., Tíscar, P.A., 2011. Buffered climate change effects in a Mediterranean
599 pine species: range limit implications from a tree-ring study. *Oecologia* 167, 847–
600 859. <https://doi.org/10.1007/s00442-011-2012-2>

601 Lloret, F., Martinez-Vilalta, J., Serra-Diaz, J.M., Ninyerola, M., 2013. Relationship
602 between projected changes in future climatic suitability and demographic and
603 functional traits of forest tree species in Spain. *Clim. Change* 120, 449–462.
604 <https://doi.org/10.1007/s10584-013-0820-6>

605 Markesteijn, L., Poorter, L., 2009. Seedling root morphology and biomass allocation of
606 62 tropical tree species in relation to drought- and shade-tolerance. *J. Ecol.* 97,
607 311–325. <https://doi.org/10.1111/j.1365-2745.2008.01466.x>

608 Matías, L., Abdelaziz, M., Godoy, O., Gómez-Aparicio, L., 2019. Disentangling the
609 climatic and biotic factors driving changes in the dynamics of *Quercus suber*
610 populations across the species' latitudinal range. *Divers. Distrib.*
611 <https://doi.org/10.1111/ddi.12873>

612 Matías, L., González-Díaz, P., Jump, A.S., 2014. Larger investment in roots in southern
613 range-edge populations of Scots pine is associated with increased growth and
614 seedling resistance to extreme drought in response to simulated climate change.
615 *Environ. Exp. Bot.* 105, 32–38. <https://doi.org/10.1016/j.envexpbot.2014.04.003>

616 Matías, L., Gonzalez-Díaz, P., Quero, J.L., Camarero, J.J., Lloret, F., Jump, A.S., 2016.
617 Role of geographical provenance in the response of silver fir seedlings to
618 experimental warming and drought. *Tree Physiol.* 36, 1236–1246.
619 <https://doi.org/10.1093/treephys/tpw049>

620 Matías, L., Jump, A.S., 2015. Asymmetric changes of growth and reproductive
621 investment herald altitudinal and latitudinal range shifts of two woody species.
622 *Glob. Chang. Biol.* 21, 882–896. <https://doi.org/10.1111/gcb.12683>

623 Matías, L., Jump, A.S., 2014. Impacts of predicted climate change on recruitment at the
624 geographical limits of Scots pine. *J. Exp. Bot.* 65, 299–310.
625 <https://doi.org/10.1093/jxb/ert376>

- 626 Matías, L., Linares, J.C., Sánchez-Miranda, Á., Jump, A.S., 2017. Contrasting growth
627 forecasts across the geographical range of Scots pine due to altitudinal and
628 latitudinal differences in climatic sensitivity. *Glob. Chang. Biol.* 23, 4106–4116.
629 <https://doi.org/10.1111/gcb.13627>
- 630 Matías, L., Zamora, R., Castro, J., 2011. Repercussions of simulated climate change on
631 the diversity of woody-recruit bank in a Mediterranean-type ecosystem.
632 *Ecosystems* 14, 672–682. <https://doi.org/10.1007/s10021-011-9437-7>
- 633 McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut,
634 J., Sperry, J., West, A., Williams, D.G., Yepez, E.A., 2008. Mechanisms of plant
635 survival and mortality during drought: why do some plants survive while others
636 succumb to drought? *New Phytol.* 178, 719–739. <https://doi.org/10.1111/j.1469-8137.2008.02436.x>
- 638 McLachlan, J.S., Hellmann, J.J., Schwartz, M.W., 2007. A Framework for Debate of
639 Assisted Migration in an Era of Climate Change. *Conserv. Biol.* 21, 297–302.
640 <https://doi.org/10.1111/j.1523-1739.2007.00676.x>
- 641 Mediavilla, S., Escudero, A., 2010. Differences in biomass allocation patterns between
642 saplings of two co-occurring Mediterranean oaks as reflecting different strategies
643 in the use of light and water. *Eur. J. For. Res.* 129, 697–706.
644 <https://doi.org/10.1007/s10342-010-0375-2>
- 645 Merlin, M., Duputié, A., Chuine, I., 2018. Limited validation of forecasted northward
646 range shift in ten European tree species from a common garden experiment. *For.*
647 *Ecol. Manage.* 410, 144–156. <https://doi.org/10.1016/j.foreco.2018.01.001>
- 648 Nicotra, A.B., Atkin, O.K., Bonser, S.P., Davidson, A.M., Finnegan, E.J., Mathesius,
649 U., Poot, P., Purugganan, M.D., Richards, C.L., Valladares, F., van Kleunen, M.,
650 2010. Plant phenotypic plasticity in a changing climate. *Trends Plant Sci.* 15, 684–
651 692. <https://doi.org/10.1016/J.TPLANTS.2010.09.008>
- 652 Nicotra, M.L., 2002. How important is natural selection? *J. Evol. Biol.* 15, 329–330.
653 <https://doi.org/10.1046/j.1420-9101.2002.0387a.x>
- 654 Padilla, F.M., Miranda, J.D., Jorquera, M.J., Pugnaire, F.I., 2009. Variability in amount
655 and frequency of water supply affects roots but not growth of arid shrubs. *Plant*
656 *Ecol.* 204, 261–270. <https://doi.org/10.1007/s11258-009-9589-0>
- 657 Parmesan, C., 2006. Ecological and Evolutionary Responses to Recent Climate Change.
658 *Annu. Rev. Ecol. Evol. Syst.* 37, 637–669.
659 <https://doi.org/10.1146/annurev.ecolsys.37.091305.110100>
- 660 Parmesan, C., Yohe, G., 2003. A globally coherent fingerprint of climate change
661 impacts across natural systems. *Nature* 421, 37–42.
662 <https://doi.org/10.1038/nature01286>
- 663 Paula, S., Ojeda, F., 2009. Belowground starch consumption after recurrent severe
664 disturbance in three resprouter species of the genus *Erica*. *Botany* 87, 253–259.
665 <https://doi.org/10.1139/B08-134>
- 666 Pérez-Harguindeguy, N., Díaz, S., Garnier, E., Lavorel, S., Poorter, H., Jaureguiberry,
667 P., Bret-Harte, M.S., Cornwell, W.K., Craine, J.M., Gurvich, D.E., Urcelay, C.,

668 Veneklaas, E.J., Reich, P.B., Poorter, L., Wright, I.J., Ray, P., Enrico, L., Pausas,
669 J.G., de Vos, A.C., Buchmann, N., Funes, G., Quétier, F., Hodgson, J.G.,
670 Thompson, K., Morgan, H.D., ter Steege, H., A van der Heijden, M.G., Sack, L.,
671 Blonder, B., Poschlod, P., Vaieretti, M. V, Conti, G., Staver, A.C., Aquino, S., C
672 Cornelissen, J.H., 2013. New handbook for standardised measurement of plant
673 functional traits worldwide. *Aust. J. Bot.* 61, 167–234.
674 https://doi.org/10.1071/BT12225_CO

675 Pérez-Ramos, I.M., Díaz-Delgado, R., de la Riva, E.G., Villar, R., Lloret, F., Marañón,
676 T., 2017. Climate variability and community stability in Mediterranean shrublands:
677 the role of functional diversity and soil environment. *J. Ecol.* 105, 1335–1346.
678 <https://doi.org/10.1111/1365-2745.12747>

679 Pérez-Ramos, I.M., Marañón, T., 2012. Community-level seedling dynamics in
680 Mediterranean forests: uncoupling between the canopy and the seedling layers. *J.*
681 *Veg. Sci.* 23, 526–540. <https://doi.org/10.1111/j.1654-1103.2011.01365.x>

682 Pérez-Ramos, I.M., Marañón, T., 2009. Effects of waterlogging on seed germination of
683 three Mediterranean oak species: Ecological implications. *Acta Oecologica* 35,
684 422–428. <https://doi.org/10.1016/J.ACTAO.2009.01.007>

685 Pérez-Ramos, I.M., Rodríguez-Calcerrada, J., Ourcival, J.M., Rambal, S., 2013a.
686 *Quercus ilex* recruitment in a drier world: A multi-stage demographic approach.
687 *Perspect. Plant Ecol. Evol. Syst.* 15, 106–117.
688 <https://doi.org/10.1016/j.ppees.2012.12.005>

689 Pérez-Ramos, I.M., Urbietta, I.R., Zavala, M.A., Marañón, T., 2012. Ontogenetic
690 conflicts and rank reversals in two Mediterranean oak species: Implications for
691 coexistence. *J. Ecol.* 100, 467–477. <https://doi.org/10.1111/j.1365-2745.2011.01912.x>

693 Pérez-Ramos, I.M., Volaire, F., Fattet, M., Blanchard, A., Roumet, C., 2013b. Tradeoffs
694 between functional strategies for resource-use and drought-survival in
695 Mediterranean rangeland species. *Environ. Exp. Bot.* 87, 126–136.
696 <https://doi.org/10.1016/j.envexpbot.2012.09.004>

697 Poorter, L., 1999. Growth responses of 15 rain forest tree species to a light gradient; the
698 relative importance of morphological and physiological traits. *Funct. Ecol.* 13,
699 396–410. <https://doi.org/10.1046/j.1365-2435.1999.00332.x>

700 Poorter, L., Markesteijn, L., 2007. Seedling Traits Determine Drought Tolerance of
701 Tropical Tree Species Author(s): Lourens Poorter and Lars Markesteijn Source:
702 *Biotropica* 40, 321–331. <https://doi.org/10.1111/j.1744-7429.2007.00380.x>

703 Quero, J.L., Villar, R., Marañón, T., Zamora, R., Vega, D., Sack, L., 2008. Relating leaf
704 photosynthetic rate to whole-plant growth: Drought and shade effects on seedlings
705 of four *Quercus* species. *Funct. Plant Biol.* 35, 725–737.
706 <https://doi.org/10.1071/FP08149>

707 R Development Core Team, 2016. R: A Language and Environment for Statistical
708 Computing. R Found. Stat. Comput. Vienna Austria.
709 <https://doi.org/10.1038/sj.hdy.6800737>

710 Ramírez-Valiente, J.A., Valladares, F., Gil, L., Aranda, I., 2009. Population differences
 711 in juvenile survival under increasing drought are mediated by seed size in cork oak
 712 (*Quercus suber* L.). *For. Ecol. Manage.* 257, 1676–1683.
 713 <https://doi.org/10.1016/j.foreco.2009.01.024>

714 Rehm, E.M., Olivas, P., Stroud, J., Feeley, K.J., 2015. Losing your edge: climate
 715 change and the conservation value of range-edge populations. *Ecol. Evol.* 5, 4315–
 716 4326. <https://doi.org/10.1002/ece3.1645>

717 Reich, P.B., Wright, I.J., Lusk, C.H., 2007. Predicting leaf physiology from simple plant
 718 and climate attributes: a global GLOPNET analysis. *Ecol. Appl.* 17, 1982–1988.
 719 <https://doi.org/10.1890/06-1803.1>

720 Saha, S., Kuehne, C., Bauhus, J., 2014. Intra- and interspecific competition differently
 721 influence growth and stem quality of young oaks (*Quercus robur* L. and *Quercus*
 722 *petraea* (Mattuschka) Liebl.). *Ann. For. Sci.* 71, 381–393.
 723 <https://doi.org/10.1007/s13595-013-0345-1>

724 Sánchez, B., García-Sánchez, A., Dios, G., 1967. *Anales de edafología y agrobiología.*
 725 Consejo Superior de Investigaciones Científicas, Instituto Nacional de Edafología
 726 y Agrobiología José María Albareda.

727 Sánchez, M.E., Caetano, P., Ferraz, J., Trapero, A., 2002. Phytophthora disease of
 728 *Quercus ilex* in south-western Spain. *For. Pathol.* 32, 5–18.
 729 <https://doi.org/10.1046/j.1439-0329.2002.00261.x>

730 Savolainen, O., Bokma, F., ... R.G.-G.-F.E. and, 2004, U., 2004. Genetic variation in
 731 cessation of growth and frost hardiness and consequences for adaptation of *Pinus*
 732 *sylvestris* to climatic changes. *For. Ecol. Manage.* 197, 79–89.

733 Savolainen, O., Pyhäjärvi, T., Knürr, T., 2007. Gene Flow and Local Adaptation in
 734 Trees. *Annu. Rev. Ecol. Evol. Syst.* 38, 595–619.
 735 <https://doi.org/10.1146/annurev.ecolsys.38.091206.095646>

736 Sniderhan, A.E., McNickle, G.G., Baltzer, J.L., 2018. Assessing local adaptation vs.
 737 plasticity under different resource conditions in seedlings of a dominant boreal tree
 738 species. *AoB Plants* 10. <https://doi.org/10.1093/aobpla/ply004>

739 Thuiller, W., 2003. BIOMOD - optimizing predictions of species distributions and
 740 projecting potential future shifts under global change. *Glob. Chang. Biol.* 9, 1353–
 741 1362. <https://doi.org/10.1046/j.1365-2486.2003.00666.x>

742 Thuiller, W., Albert, C., Araújo, M.B., Berry, P.M., Cabeza, M., Guisan, A., Hickler,
 743 T., Midgley, G.F., Paterson, J., Schurr, F.M., Sykes, M.T., Zimmermann, N.E.,
 744 2008. Predicting global change impacts on plant species' distributions: Future
 745 challenges. *Perspect. Plant Ecol. Evol. Syst.* 9, 137–152.
 746 <https://doi.org/10.1016/J.PPEES.2007.09.004>

747 Vessella, F., López-Tirado, J., Simeone, M.C., Schirone, B., Hidalgo, P.J., 2017. A tree
 748 species range in the face of climate change: cork oak as a study case for the
 749 Mediterranean biome. *Eur. J. For. Res.* 136, 555–569.
 750 <https://doi.org/10.1007/s10342-017-1055-2>

751 Wright, I.J., Reich, P.B., Westoby, M., Ackerly, D.D., Baruch, Z., Bongers, F.,

752 Cavender-Bares, J., Chapin, T., Cornelissen, J.H.C., Diemer, M., Flexas, J.,
753 Garnier, E., Groom, P.K., Gulias, J., Hikosaka, K., Lamont, B.B., Lee, T., Lee, W.,
754 Lusk, C., Midgley, J.J., Navas, M.-L., Niinemets, Ü., Oleksyn, J., Osada, N.,
755 Poorter, H., Poot, P., Prior, L., Pyankov, V.I., Roumet, C., Thomas, S.C., Tjoelker,
756 M.G., Veneklaas, E.J., Villar, R., 2004. The worldwide leaf economics spectrum.
757 Nature 428, 821–827. <https://doi.org/10.1038/nature02403>

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Table 1: Results from linear models (F-statistics and *P*-value) explaining trait variations across experimental treatments (provenance, Pr; watering, W) of the morphological traits obtained after the watering period.

	Provenance		Watering		Pr × W	
	F	<i>P</i>	F	<i>P</i>	F	<i>P</i>
<i>Morphological traits</i>						
Total biomass	0.04	0.85	0.42	0.66	0.29	0.75
Shoot length	4.20	0.043	5.30	0.006	0.21	0.81
Root length	7.75	0.006	1.57	0.21	0.91	0.41
R:S	38.79	<0.0001	8.84	0.0003	0.97	0.38
LMR	8.32	0.05	4.66	0.01	0.02	0.79
SMR	26.80	<0.0001	2.50	0.087	0.14	0.87
SLA	6.54	0.011	6.55	0.002	0.52	0.60
SSL	8.70	0.004	0.86	0.43	0.67	0.51
<i>df</i>	1		2		2	

Abbreviations: R:S, root to shoot ratio; LMR, leaf mass ratio; SMR, stem mass ratio; SLA, specific leaf area; SSL, specific stem length.

Figure 1: Responses of several key morphological traits (shoot length, panel a; specific leaf area, panel b; root:shoot ratio, panel c) to experimental treatments (provenance: north, south; watering: high, medium, low) after the watering period. Different letters

770 denote differences among watering levels within the same provenance after Bonferroni
771 post hoc test. Error bars represent $\pm$ SE.

Figure 2: Differences in leaf relative water content (LRWC, in %) from the onset of the extreme drought period across provenances (panel a) and watering levels (panel b). Significant differences at $P < 0.05$ are indicated with an asterisk. Error bars represents $\pm$ SE.

Figure 3: Mean photosynthetic rate (A_{max}) of seedlings from the two provenances (panel a, watering levels pooled) growing under the different watering levels (panel b, provenances pooled). Vertical red line represents the onset of the extreme drought period. Error bars represents standard error terms. Significant differences within measuring date are indicated by asterisks (* < 0.05; ** < 0.001; *** < 0.0001).

Figure 4: Variations in the leaf C:N ratio (panel a) and C isotopic discrimination (panel b) across experimental treatments at the end of the extreme drought period. Different letters denote differences among watering levels within the same provenance after Bonferroni post hoc test. Error bars represents $\pm$ SE.

Figure 5: Correlations between root to shoot ratio (R:S) and survival time since the onset of the extreme drought period (in days). Northern provenance (N) is represented with circles and southern (S) with triangles. High watering level is indicated in black, medium in grey and low in white. Overall correlation $R^2 = 0.35$ and $P < 0.0001$.

Table S1: Main characteristics of the populations used as seed source for the experiment.

	North	South
Provenance	Sobreiral do Arnego	Tazekka National Park
Location	NW Spain	N Morocco
Latitude	42° 45' 59" N	34° 02' 40" N
Longitude	8° 05' 40" W	4° 11' 45" W
Altitude (m a.s.l.)	359	1440
Annual precipitation (mm)	1214	433
Mean annual temperature (°C)	13.4	13.7
Tree density (ind. ha ⁻¹)	336	1080
Basal area (m ² ha ⁻¹)	83.1	69.2

796 **Table S2:** Mean values $\pm$ SE of the measured traits. Morphological traits were measured
797 at the end of the watering period, while chemical traits (leaf C, N, C:N and $\delta^{13}\text{C}$) were
798 obtained at the end of the extreme drought period.

	Provenance		Watering		
	North	South	High	Medium	Low
Total biomass (g)	6.6 $\pm$ 0.2	6.7 $\pm$ 0.3	6.6 $\pm$ 0.3	6.9 $\pm$ 0.3	6.5 $\pm$ 0.3
Shoot length (cm)	33.9 $\pm$ 1.6	29.9 $\pm$ 1.3	29.2 $\pm$ 2.0	37.0 $\pm$ 1.9	31.0 $\pm$ 1.5
Root length (cm)	56.7 $\pm$ 1.4	63.2 $\pm$ 1.9	61.1 $\pm$ 2.3	61.8 $\pm$ 2.0	56.9 $\pm$ 1.8
R:S	1.3 $\pm$ 0.1	1.7 $\pm$ 0.1	1.7 $\pm$ 0.1	1.3 $\pm$ 0.1	1.4 $\pm$ 0.1
LMR (g g ⁻¹)	0.31 $\pm$ 0.0	0.27 $\pm$ 0.0	0.27 $\pm$ 0.0	0.32 $\pm$ 0.0	0.30 $\pm$ 0.0
SMR (g g ⁻¹)	0.13 $\pm$ 0.0	0.10 $\pm$ 0.0	0.11 $\pm$ 0.0	0.12 $\pm$ 0.0	0.12 $\pm$ 0.0
SLA (m ² kg ⁻¹)	113.0 $\pm$ 2.0	107.6 $\pm$ 1.3	110.3 $\pm$ 1.5	105.5 $\pm$ 1.3	115.8 $\pm$ 3.0
SSL (cm g ⁻¹)	40.9 $\pm$ 1.6	48.3 $\pm$ 1.9	42.5 $\pm$ 2.3	47.1 $\pm$ 2.3	43.4 $\pm$ 2.0
Leaf C:N	47.1 $\pm$ 4.5	44.9 $\pm$ 4.2	62.1 $\pm$ 4.8	45.5 $\pm$ 2.7	30.6 $\pm$ 1.7
Leaf C	46.5 $\pm$ 0.5	46.0 $\pm$ 0.4	45.7 $\pm$ 0.6	46.2 $\pm$ 0.3	46.9 $\pm$ 0.6
Leaf N	1.14 $\pm$ 0.13	1.14 $\pm$ 0.09	0.78 $\pm$ 0.07	1.05 $\pm$ 0.07	1.58 $\pm$ 0.1
$\delta^{13}\text{C}$ (‰)	-28.2 $\pm$ 0.2	-28.4 $\pm$ 0.2	-28.7 $\pm$ 0.2	-28.2 $\pm$ 0.1	-27.9 $\pm$ 0.2

799 Abbreviations: R:S, root to shoot ratio; LMR, leaf mass ratio; SMR, stem mass ratio;
800 SLA, specific leaf area; SSL, specific stem length.

801

Table S3: Results from the rm-ANOVA analyses explaining variations in leaf relative water content (LRWC) and maximum leaf-level photosynthesis (A_{max}) across provenance (Pr) and watering (W) treatments during the extreme drought period (experimental weeks 22-28 for LRWC and 22-27 for A_{max}). Variation across time (T) was analysed as a within-subject factor. Significant P -values are indicated in bold.

Trait	Factor	F	P	df_{Num}	df_{Den}
LRWC	<i>Between subjects</i>				
	Provenance (Pr)	0.01	0.96	1	24
	Watering (W)	2.74	0.08	2	24
	Pr $\times$ W	1.38	0.27	2	24
	<i>Within subjects</i>				
	Time (T)	10.34	<0.0001	6	19
	T $\times$ Pr	0.64	0.69	6	19
	T $\times$ W	0.81	0.64	12	38
	T $\times$ Pr $\times$ W	1.11	0.38	12	38
A_{max}	<i>Between subjects</i>				
	Pr	0.06	0.82	1	14
	W	5.04	0.02	2	14
	Pr $\times$ W	0.40	0.68	2	14
	<i>Within subjects</i>				
	T	65.52	<0.0001	7	8
	T $\times$ Pr	1.64	0.25	7	8
	T $\times$ W	1.47	0.23	14	16
	T $\times$ Pr $\times$ W	0.13	0.99	14	16

809

810 **Figure S1:** Soil volumetric water content (VWC, in %) recorded before (week 6),
811 during the watering period (weeks 8 to 20, panel a) and during the extreme drought
812 period (weeks 21 to 45, panel b). Significant differences across watering levels (high,
813 medium and low) within measuring dates are indicated by asterisks.

Figure S2: Survival probabilities from the onset of the extreme drought period of *Q. suber* seedlings from northern (black line) and southern (grey line) provenances under the different watering levels (high, medium, low). Differences between provenances (χ^2 and *P*-values) after a Log-Rank test are indicated for each watering level.

Appendix A: Determination of physiological and morphological traits.

1.1 Photosynthesis: Maximum leaf-level photosynthetic capacity (A_{max}) was measured using a LI-6400xt infrared gas-exchange analyser system (Li-Cor Inc., Lincoln, NE, USA). The measurements took place between 10:00 h and 14:00 h using the 2×3 LED with the following settings chamber: 400 ppm reference CO₂ concentration, 1500 $\mu\text{mol m}^{-2} \text{s}^{-1}$ light-saturating photosynthetic photon flux density, block temperature at 25 °C to match ambient air temperature, and relative humidity on full scrub. To determine projected leaf area of the measured leaves, they were further scanned and processed using the Image Pro Plus software (Media Cybernetics, MD, USA), and this value was used to correct A_{max} .

1.2 Carbon, nitrogen and isotopic discrimination: For the determination of C and N content in leaves and the relative abundance of ¹³C, leaves were ground to powder and total carbon and nitrogen as well as the bulk isotopic composition were analysed by dry combustion in a Flash 2000 elemental micro-analyser coupled via ConFlo IV interface to a Delta V Advantage isotope ratio mass spectrometer (Thermo Scientific, Bremen, Germany). Isotopic ratios are reported as parts per thousand deviations (expressed as δ values) referred to appropriate IAEA standards (VPBD):

$$\delta = \left[\frac{R_{\text{sample}} - R_{\text{standard}}}{R_{\text{standard}}} \right] \times 1000 \quad \text{Eq. (A1)}$$

where R is the ¹³C/¹²C ratio. The standard deviations of $\delta^{13}\text{C}$ were typically less than ± 0.05 ‰. The total C and N values were used for the calculation of the leaf C:N ratio for each experimental treatment.

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